Egyptian Journal of Aquatic Research (2012) **38**, 233–239

National Institute of Oceanography and Fisheries

Egyptian Journal of Aquatic Research

http://ees.elsevier.com/ejar
www.sciencedirect.com

FULL LENGTH ARTICLE

In vitro: Antimicrobial potential and phytochemical screening of some egyptian aquatic plants

Said M. Daboor [a,b,*], Amany M. Haroon [a]

[a] *Hydrobiology Laboratory, National Institute of Oceanography and Fisheries, Cairo, Egypt*
[b] *Medical Bio-Sciences Department, Al-Farabi College, Riyadh, KSA*

Received 4 December 2012; accepted 26 December 2012
Available online 16 February 2013

KEYWORDS

Phytochemical;
Macrophytes extracts;
Antimicrobial activates;
MIC;
MBC

Abstract Ethanolic and water extracts of three macrophytes (roots); *Echinochloa stagnina* (*Retz*.) P. Beauv; *Pistia stratiotes* (L.) and *Nymphaea lotus* (L.) from El-Serw, Dakahlia, Egypt were studied to verify their antimicrobial activities against certain microbial isolates by using agar well diffusion method. Bi-fold dilution technique revealed that all the tested microorganisms showed susceptibility to moderate resistance against *E. stagnina* ethanolic extract, with minimum inhibitory concentrations (MICs) values varied between 78.125 and 625.00 μg/ml, while its water extract showed antimicrobial activities that were significantly reduced by almost 50% compared to the ethanolic one. On the other hand *N. lotus* and *P. stratiotes* water extracts had no activity against all tested microorganisms. The phytochemical screening revealed the presence of some biologically active substances (flavonoides, tannins, sterols and resins) with different concentrations. *E. stagnina* roots were shown to possess the highest polyphenols contents 3.20 ± 0.01 g/100 g dry wt., moreover some heavy ions as Fe, Mn, Zn and Cu were presist with different concentrations varied significantly ($P < 0.05$) with plant species and solvent used in extraction.

1. Introduction

A dramatic increase in microbial antibiotic resistance has developed over the last forty years in both the agriculture and medical sectors, this increasing forced the researchers to develop a new antimicrobial drugs not based on the synthetic agents for controlling pathogenic species (Pai et al., 2004; Shah, 2005; Ibrahim et al., 2011; Obeidat et al., 2012). Thus, demand has increased for less harmful and environmentally friendly natural products, especially that produced by plants, which are sources for novel bioactive substances. During the period 2000–2006 almost half of the new extracted natural products were used to control most of the infectious diseases demonstrated their value as drugs (Newman and Cragg, 2007).

Various aquatic plants have the ability to produce bioactive materials that showed antibacterial activities (Abu Ziada et al., 2008; Fareed et al., 2008; Haroon et al., 2009; Sridevi et al., 2010), antifungal activities (Bhosale et al., 1999; Haroon, 2006), antiviral activities (Verma et al., 2008; Shin et al.,

* Corresponding author at: 101 Al Kasser Al Ani Street, 14th floor, Cairo, Egypt.
E-mail address: Saiddaboor@yahoo.ca (S.M. Daboor).
Peer review under responsibility of National Institute of Oceanography and Fisheries.

ELSEVIER Production and hosting by Elsevier

1687-4285
http://dx.doi.org/10.1016/j.ejar.2013.01.005

2010; Sohail et al.,2011) and also antialgal activities (Li and Hu, 2005; Yi et al., 2012). For our knowledge few studies concerned the antimicrobial activities of *Echinochloa stagnina* (*Retz*.) P. Beauv, *Nymphaea lotus* (L.) and *Pistia stratiotes* (L.) that isolated from the Nile Delta (Egypt) Region, especially their roots extracts, except the results reported by Abu Ziada et al. (2008) and Yisa (2009) regarding the antimicrobial activities of *N. lotus* (L.) and *P. stratiotes* (L.) leaves and whole plants. However, neither the antimicrobial activities nor the phytochemical screening of *E. stagnina* (*Retz*.) P. Beauv, *N. lotus* (L.) and *P. stratiotes* (L.) roots were evaluated. This work made an attempt for the first time to test and compare the antimicrobial activities, in addition to the phytochemical screening of *E. stagnina* (*Retz*.) P. Beauv, *N. lotus* (L.) and *P. stratiotes* (L.) roots extracts.

2. Materials and methods

2.1. Plant materials

The tested plants, *E. stagnina* (*Retz*.) P. Beauv; *P. stratiotes* (L.) and *N. lotus* (L.) were collected from two different water sources at El-Serw village, south of Manzalah Lake, north eastern Egypt (longitude 31°: 45′–32°–50′ E and latitude 31°: 00–31°: 35′ N), and identified according to Täckholm (1974) and Pandey (1982). In addition, these plants were identified depending on a comparative materials available in the Herbarium and revised by Professor Dr. Kamal Shaltout (Kshaltout@yahoo.com); voucher specimens are kept in the Herbarium of the Botany Department, Faculty of Science, Tanta University (TANE) Egypt, *N. lotus* (L.) (No. 3161, 3162), *P. stratiotes* (L.) (No. 3163, 3164) and *E. stagnina* (*Retz*.) P. Beauv (No. 3165, 3166).

2.1.1. Preparation of extracts

Roots of the selected plants were separated, washed with tap water followed by distilled sterilized water to remove all debris and unwanted associated parts, dried in an oven (Heraeus, Modle VT6025) at 50 ± 2 °C to constant weight, and ground with a blender until get fine powder (Bushmann and Ailstock, 2006). All steps are illustrated in Fig. 1.

The air-dried powdered samples were extracted with both, ethanol and bidistilled water following the same method described by (Ali-Shtayeh et al., 1998). 20 g dried powdered roots were infused in 200 ml of bidistilled water or 95% ethanol, allowed to stand for 72 h. at room temperature (≈35 °C), filtered through Whatman No. 1 filter paper and concentration using a rotate evaporator at 45 ± 2 °C.

2.2. Phenolic contents and phytochemical screening

Folin–Ciocalteu method (Meda et al., 2005) was used to determine the total phenolic content of the plant extracts and the data were expressed as gram gallic acid equivalents (GAE)/ 100 g dry matter from plant samples. The preliminary phytochemical screening for, flavonoides, tannins, sterols, resins and mucilage was assessed following the methodology of (Harborn, 1998; Kokate, 2001).

2.3. Elementary analysis

The elementary analysis takes place in all prepared plants extracts using Pertkin Elmer 2100 Flame Atomic Absorption Spectrophotometer with Auto- sampler (Allen et al., 1986).

2.4. Antimicrobial activity assay

2.4.1. Tested microorganisms

Inhibitory activities of the tested extracts were evaluated against eight microbial isolates including, *Bacillus subtilis* and *Staphylococcus aureus* (Gram positive bacteria), *Escherichia coli*, *Kelbsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella* sp. and

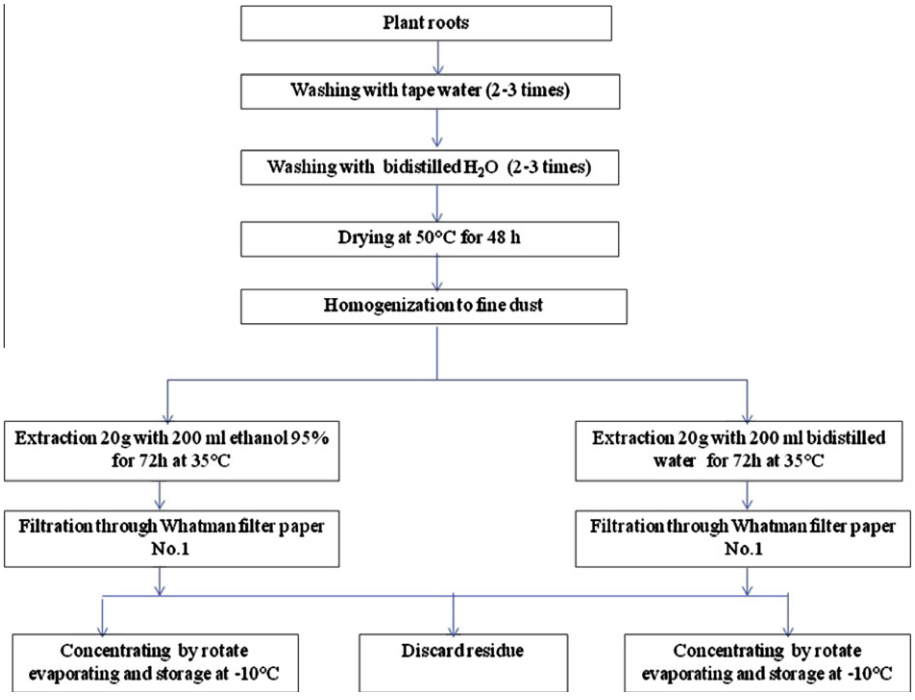

Figure 1 Plant's root preparation and extraction.

Shigella sp. (Gram negative bacteria) and one yeast; *Candida albicans* ATCC10231. All these microbial isolates (except ATCC10231) were ecological relevant to the tested plants (Haroon and Daboor, 2009) and identified based on Bergey's Manual of determinative Bacteriology (Holt et al., 1994).

2.4.2. Antimicrobial screening

The agar well diffusion method recommended by the National Committee for Clinical Laboratory Standards (NCCLS), 1999 and used by several researchers such as Traub et al. (1998), Daboor (2001), Okeke et al. (2001), Candan et al.(2003) and Salvador et al. (2007) were used for the antimicrobial screening. Five ml of a fresh growth (12 h old culture, with 10^6 CFU ml^{-1} at wavelength λ620 nm) of each isolate was mixed well with 100 ml melted warm (≈45 °C) Mueller Hinton Agar (Oxoid-CM 337) for bacterial isolates and Sabouraud dextrose agar (Oxoid-CM41) media for yeast isolate. The concentrated plant extracts were redissolved in each solvent to a final concentration of 0.2 g ml^{-1} and filtrated through 0.45 µ filter. Each agar well (5.0 mm diameter) was filled with 100 µl of each extract (20 mg); each plate contained three extracts in addition to one negative control (100 µl of solvent). The plates were hold at 4 °C for two h followed by incubation at 33 ± 2 °C for 24–48 h. The clearance zones (inhibition zones mm) including the well diameter were recorded after subtraction of that produced by the solvent, clearance zones having >6 mm were considered as positive results.

2.4.3. Determination of minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs)

Minimum inhibitory concentrations (MIC) were detected by the broth dilution methods described by NCCLS (2009). Two fold dilution of *E. stagnina* ethanolic extract mad with Mueller Hinton broth (MHB; OXOID-CM405) for bacterial isolates and Sabouraud dextrose broth (SDB; DIFCO) for yeast. An overnight old culture of the tested strains were suspended in MBH with optical density 10^6 CFU/ml (verified by plat counts). An aliquot of 100 µl was mixed with each concentration; one negative blank tube was inoculated with the same quantity of the solvent and one positive tube without the extraction. The MIC of positive control antibiotics (Streptomycin and Nystatin) was individually determined in parallel experiments in order to control the sensitivity of the test organisms (Özbay and Alim, 2009).

All tubes were incubated at 33 ± 2 °C for 24–48 h. The lowest concentration that did not show any visible growth (at wavelength λ 620 nm) compared to the blank was considered as the MICs. All tubes that did not show any change of optical density compared to the control (no visible growth) were cultured on NA, YPM agar and blood agar (Sigma) and incubated at 33 ± 2 °C for 24–48 h, the MBCs and MFCs were evaluated according to Yu et al. (2004) and Ratnam and Raju (2008). To confirm the results, two reference standard antibiotics, Strptomycine and Nystatin were used as standard antibiotics.

3. Statistical analysis

Data are presented as mean ± SE and analyzed by one-way ANOVA to assess the significance of variation in total phenols content in relation to plant species using SAS program (1996), and bioactivities of the extracts (n = 3) were analyzed by two-way ANOVA with 95% confidence limits ($P < 0.05$), using Minitab 15 (for Dalhousie University, Halifax, NS, Canada) for windows statistical package (Ryan et al., 1976).

4. Results and discussion

4.1. Phenolic contents and phytochemical screening

The total phenol contents in roots extracts of *E. stagnina* (*Retz.*) P. Beauv, *P. stratiotes* (L) and *N. lotus* showed a significant difference with plant species ($P < 0.05$) (Table 1). *E. stagnina* was recorded to have the highest total phenolic content (3.20 ± 0.01 g/100 g), followed by *N. lotus* and *P. stratiotes* (2.58 ± 0.0 and 2.42 ± 0.01 g/100 g, respectively).

The preliminary phytochemical screening revealed the presence of flavonoides, tannins, sterols and resins in *N. lotus* extract. However, sterols and resin were detected within *P. stratiotes* extract and sterols and tannins were found within *E. stagnina* extract (Table 1).

4.2. Elementary analysis

The elemental analysis of the tested plants roots extracts, revealed the presence of Fe, Mn, Zn and Cu ions with a significant difference ($P < 0.05$) affected with both the plant species and the extraction solvent (Table 2). Among the studied plants *P. stratiotes* was shown to possess the highest amounts of Fe, Mn and Zn within its ethanolic extract, whereas *E. stagnina* possess the highest amounts of Cu (123.1 ± 0.0 and 76.1a ± 0.05 µg/g) in both ethanolic and aqueous extract respectively.

4.3. Antimicrobial activities of the tested plants

Antimicrobial activities of the plants extracts (ethanolic and water solutions) *in vitro* showed inhibitory activities against

Table 1 Phytochemical screening of *Nymphaea lotus*, *Pistia stratiotes* and *Echinochloa stagnina*, extracts.

Plants (roots)	Phytochemical materials					
	Phenols (g/100 g)*	Flavonoids**	Tannins**	Sterols**	Resin**	Mucilage**
N. lotus	2.58[b] ± 0.0	+ ve	+ + + ve	+ + ve	+ ve	ND
P. stratiotes	2.42[c] ± 0.01	ND	ND	+ + ve	+ ve	ND
E. stagnina	3.20[a] ± 0.01	ND	+ + ve	+ + ve	ND	ND

* Mean value ± SD (n = 3), expressed as gram gallic acid equivalent/100 g dry weight., values with the same letters within the column are not significantly different at ($P < 0.05$).

** ND; not detected, + ve; small concentration, + + ve; moderate concentration, + + + ve; high concentration.

Table 2 Elements concentrations in the studied plants extracts expressed on dry weight basis.

Element	Extract	Plant		
		P. stratiotes	*N. lotus*	*E. stagnina*
Fe (mg/g)	E	$54.10^{a} \pm 0.06$	$42.24^{b} \pm 0.02$	$37.52^{c} \pm 0.01$
	A	$10.73^{b} \pm 0.0$	$10.11^{c} \pm 0.0$	$24.24^{a} \pm 0.03$
Mn (mg/g)	E	$142.43^{a} \pm 0.07$	$92.37^{b} \pm 0.07$	$90.07^{c} \pm 0.03$
	A	$96.24^{b} \pm 0.0$	$92.46^{c} \pm 0.06$	$137.46^{a} \pm 0.06$
Zn (μg/g)	E	$12.23^{a} \pm 0.02$	$2.03^{c} \pm 0.03$	$3.4^{b} \pm 0.06$
	A	$2.94^{c} \pm 0.0$	$5.1^{b} \pm 0.06$	$8.4^{a} \pm 0.06$
Cu (μg/g)	E	$16.24^{b} \pm 0.0$	$14.1^{c} \pm 0.06$	$123.1^{a} \pm 0.06$
	A	$10.74^{c} \pm 0.0$	$11.48^{b} \pm 0.0$	$76.1^{a} \pm 0.05$

E = ethanolic extract and A = aqueous extract.
Different upper case letters in the same row indicate significant differences ($P < 0.05$).

some Gram positive bacteria, Gram negative bacteria, and yeast with significant effects depending not only on the tested microorganisms but also on the extraction solvent. The extracts showed antimicrobial activities with a significantly differences ($f = 90.64$, df = 2, $P < 0.05$), where *E. stagnina* ethanolic extract found to posses antimicrobial activities exhibited a clear zones with *Staphylococcus aureus*, *Candida albicans* and *Bacillus subtilis* with values within the range of 19.333 ± 1.155 to 19.667 ± 0.577 mm, without significant difference (Fig. 2, histogram). However, these clearance zones were reduced significantly with *Escherichia coli*, *Kelbsiella pneumoniae*, *Pseudomonas aeruginosa*, *Shigella* sp. and *Salmonella* sp. with values of 12.667 ± 0.577–16.667 ± 1.527 mm, where *Salmonella* and *Shigella* spp. were the most resistant isolates ($f = 19.18$, df = 7, $P < 0.05$) recorded the lowest inhibition zones ranged between 12.667 ± 0.577 and 13.667 ± 1.547 mm, without a significant difference followed by *K. pneumoniae* and *E. coli* showed a moderate resistance with insignificantly clearance zones of the values of 15.667 ± 0.577–16.333 ± 1.527 mm. On the other hand, ethanolic extracts for *P. stratiotes* and *N. lotus* showed similar inhibitory effects for both *B. subtilis* and *E. coli* without any significant difference with inhibition zones of values between 16.133 ± 0.115 and 16.30 ± 0.10 mm (Fig. 2, histogram).

Some research papers showed that *S. aureus* and *Bacillus* were the most sensitive isolates to the estuarine submersed aquatic plants extracts, and that due to the cell wall structure of Gram positive bacteria that consists of a single layer, while Gram negative bacteria cell wall have a double layer membrane surrounding the bacterial cell, that make cell membrane not permeabilized to the antimicrobial agent(s) and hence delay the osmotic lysis of a bacterial cell and membrane permeabilization led to resistance the ethanolic effect (Bushmann and Ailstock, 2006; Wang et al., 2007).

Water extract of *E. stagnina* showed antimicrobial activities that were significantly reduced by almost 50% compared to the ethanolic one, it did not affect the tested Gram positive bacteria, while the inhibition zones with the tested Gram negative bacteria and *C. albicans* ranged from 6.833 ± 0.058 to 9.0 ± 0.1 mm (Fig. 2, diagram). For the water extracts of *P. stratiotes* and *N. lotus* no inhibitory activity were found against all the tested microorganisms (Fig. 2, diagram), and that due to the relatively low capacity of most bioactive compounds to dissolve in water. Similar results have been found by Akinsulire et al. (2007) and Kumar et al. (2008), where they discovered, the antibacterial activities of organic solvents extracts of seagrsses were more active than that of water extracts. It is clear that ethanol appeared better as an extractant, judging

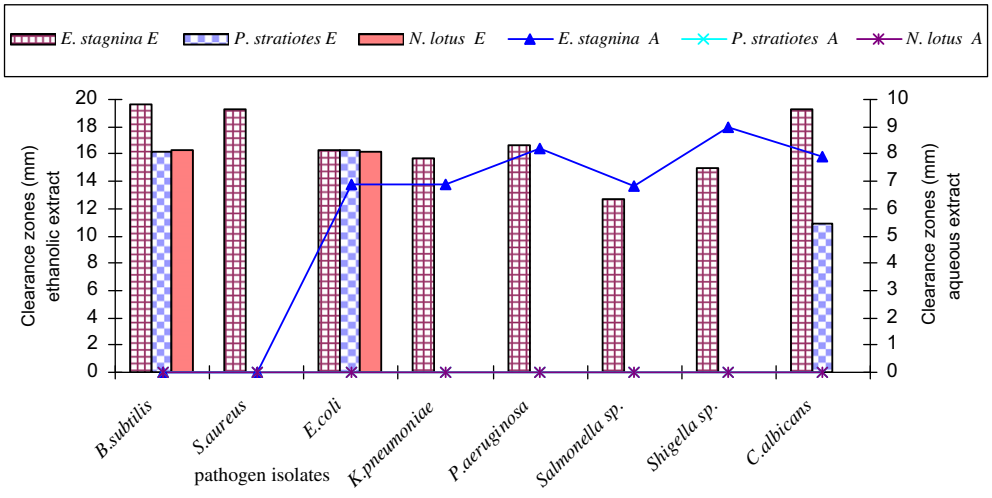

Figure 2 Antimicrobial activities of ethanolic (E) and aqueous (A) extracts of *Echinochloa stagnina*, *Pistia stratiotes* and *Nymphaea lotus*. Mean value ± SD, $n = 3$(clearance zones of inhibition (in millimeter) including well of 5 mm in diameter).

Table 3 Minimum inhibitory concentrations and minimum bactericidal concentrations (μg/ml).

Tested microorganisms	MIC and MBC			
	Ethanolic extract		Streptomycin[a]	Nystatin[b]
	MIC	MBC/MFC	MBC	MFC
B. subtilis	156.250	250.00	2.00	NT
S. aureus	156.250	220.00	6.00	NT
E. coli	312.500	380.00	10.00	NT
K. pneumoniae	312.500	360.00	12.00	NT
P. aeruginosa	312.500	390.00	4.00	NT
Salmonella sp.	625.000	710.00	20.00	NT
Shigella sp.	312.500	500.00	18.00	NT
C. albicans ATCC10231	78.125	120.00	NT	12.00

MIC: minimum inhibitory concentrations, MBC: minimum bactericidal concentrations, MFC: minimum fungicidal concentration, NT: not tested, ATCC: American Type Culture Collection, a: antibacterial, b: antifungal.

from the wider activity spectrum and the resultant effect on the tested isolates, this is due to the great ability of alcohol to solublize and extract some active compounds such as: phenolic compounds, saponins, tannins, and flavonoids is greater than water (Akinsulire et al., 2007; Yisa, 2009), some compounds of these groups are known to be either bactreiostatic or bactericidal materials, based on their concentration (Dorman and Deans, 2000). Many researchers (Soetan et al., 2006; Yisa, 2009) reported that flavonoids and phenolic derivatives have antimicrobial activities; these compounds are believed to function by affecting the bacterial cell membrane integrity, resulted in inhibition the bacterial growth (Trombetta et al., 2005; Hendrich, 2006).

Regarding the role of elements in living organisms many metals and metalloids (e.g., Zn, Cu, Mn) are essential in the functioning of living organisms as micronutrients serving as structural proteins and pigment, used in the redox processes, regulation of the osmotic pressure, maintaining the ionic balance and enzyme component of the cells (Kosolapov et al., 2004). Though, the present work involved estimation of Fe, Mn, Zn and Cu in the used extracts. Where, the results showed the variation in element content with plant species and solvent used in extraction.

Iron being essential for all the micro-organisms whose acquisition is done by the synthesis of siderophores which makes it possible to transfer it inside the bacterial cell (Expert, 1999). As recorded by Chung et al. (1998), the inhibition of the growth of the intestinal bacteria (*Bacteroides fragilis*, *Clostridium perfringens*, *E. coli* and *Enterobacter cloacae*) by the tannic acid is probably related to the high capacity of the latter to fix iron. Though, it has been established that the presence of Fe ion in both *P. stratiotes* and *N. lotus* ethanolic extracts at higher concentrations compared to *E. stagnina* extract may be one of the reasons that decreasing their antimicrobial activity.

Regarding the effect of Cu, Zn and Mn on bacterial growth, (Otludil et al., 2005) recorded that bacterial growth was stimulated greatly in medium which contains Zn and Mn with respect to control. However, bacterial growth was effectively inhibited in other metal ions such as Ni and Cu with respect to control. So, in the present work the presence of Zn and Mn at lower concentrations and Cu at higher concentration in *E. stagnina* ethanolic and aqueous extracts could be another reason of its greater antimicrobial activity compared to the other two plant species. In comparing our results with the finding of Abu Ziada et al., 2008; Yisa, 2009 for antimicrobial activity of *P. stratiotes* and *N. lotus*, the lowest antimicrobial activity recorded in the present investigation could be related to the presence of active substances in higher concentrations in these plants leaves compared by the roots.

4.4. Minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs)

The data in (Table 3) represented the MICs values of the *E. stagnina* ethanolic extract. These values were varied between 78.125–625.00 μg/ml, while *Salmonella* sp. showed the maximum resistance with the highest MIC value of 625.00 μg/ml, meanwhile the lowest MIC value was recorded for *C. albicans* (78.125 μg/ml) suggested that a very small amount of the crude extract is required to suppress *C. albicans*. MBC/minimum fungicidal concentration (MFC) values revealed that *C. albicans* was completely inhibited with the minimum amount of the extract showed a MFC value of 120 μg/ml, that mean the ethanolic extract of *E. stagnina* is very potent against *C. albicans*. The probable mechanism of plant(s) extracts that suppress yeast is that the bioactive compound(s) integrated with *C. albicans* cell wall resulted in changing the cell membrane permeability and a disruption of nutrients and wastes crossing the membrane was occurred, then the bioactive material(s) diffused inside the cell, meantime cell membrane and phospholipids of the cell nucleus are combined together resulting in damage of cell functions as well as structures and the cell organ disappearance (Donlan and Costerton, 2002; Kong et al., 2009).

5. Conclusions

The present work proved that the three tested plants did inhibitory activity against some tested microorganisms varied significantly with plant species and solvent used in extraction. In addition, the results suggested that, the studied aquatic plants extracts possess some natural compounds that could be used as antimicrobial agents. The nature and number of these active component(s) are not clear, however it still worthwhile to take into account the use of these bioactive materials as antimicrobial agents. Hence these compounds should be investigated for natural antimicrobial agents and more studies should be carried out to purify and identify these compound(s).

Acknowledgement

We express our gratefulness to Dr. Suzanne M. Budge, Associate Professor at Dalhousie University, Halifax, Nova Scotia, Canada, for her continuous support.

References

Abu Ziada, E., Mashaly, A., Abd El-Monem, M., Torky, M., 2008. Economic potentialities of some aquatic plants growing in north east Nile Delta. Egypt Journal of Applied Science 8, 1395–1405.

Akinsulire, R., Aibinu, E., Adenipekun, T., Adelowotan, T., Odugbemi, T., 2007. *In vitro* antimicrobial activity of crude extracts from plants *Bryophyllum pinnatum* and *Kalanchoe crenata*. African Journal of Traditional, Complementary and Alternative Medicines 3, 338–344.

Ali-Shtayeh, S., Yaghmour, R., Faidi, R., Salem, K., Al-Nuri, A., 1998. Antimicrobial activity of 20 plants used in folkloric medicine in the palestinian area. Journal of Ethnopharmacology 60, 265–271.

Allen, S.E., Grimshow, H.M., Parkinson, J.A., Quarmby, C., Roberts, J.D., 1986. Chemical analysis. In: Chapman, S.B. (Ed.), Methods in Plant Ecology. Blackwell Scientific Publications, Oxford, London, pp. 411–416.

Bhosale, S.H., Jagtap, T.G., Naik, C.G., 1999. Antifungal activity of some marine organisms from India, against food spoilage *Aspergillus* strains. Mycopathologica 147, 133–138.

Bushmann, P.J., Ailstock, M.S., 2006. Antibacterial compounds in estuarine submersed aquatic plants. Journal of Experimental Marine Biology and Ecology 331, 41–50.

Candan, F., Unlu, M., Tepe, B., Daferera, D., Polissiou, M., Sokmen, A., Akpulat, K., 2003. Antioxidant and antimicrobial activity of the essential oil and methanol extracts of *Achillea millefolium* subsp. *millefolium* Afan (Asteraceae). Journal of Ethnopharmacology 87, 215–220.

Chung, K.T., Wong, T.Y., Wei, C.I., Huang, Y.W., Lin, W., 1998. Tannins and human health: a review. Critical Reviews Food Science Nutrition 38, 421–464.

Daboor, S.M., 2001. Pathological and biochemical studies on microorganisms isolated from faba bean (unpublished, Master's thesis). Botany Department, Faculty of science, Benha University, Benha, Egypt.

Donlan, R.M., Costerton, J.W., 2002. Biofilms: survival mechanisms of clinically relevant microorganisms. Clinical Microbiology Reviews 15, l67–l193.

Dorman, H.J., Deans, S.G., 2000. Antimicrobial agents from plants: antimicrobial activity of plant volatile oils. Journal of Applied Microbiology 88, 308–316.

Expert, D., 1999. With holding and exchanging iron: interactions between *Erwinia* spp and their plant hosts. Annual Review of Phytopathology 37, 307–334.

Fareed, M.F., Haroon, A.M., Rabh, S.A., 2008. Antimicrobial activity of some macrophytes from Lake Manzalah (Egypt). Pakistan Journal of Biological Sciences 11 (21), 2454–2463.

Harborn, J.B., 1998. Phytochemical methods. A guide to modern techniques of plant analysis, third ed. Chapman and Hall Int., London, UK.

Haroon, A.M., 2006. Effect of some macrophytes extracts on growth of *Aspergillus parasiticus*. Egyptian Journal of Aquatic Research 32, 301–313.

Haroon, A.M., Daboor, S.M., 2009. The role of different macrophytes groups in water quality, sediment chemistry and microbial flora of both irrigation and drainage canals. World Applied Sciences Journal 6, 1221–1230.

Haroon, A.M., Sharshar, K., Fareed, M., 2009. Investigation on *Vibrio* sp. isolated from diseased caryfish (*Procambarus calarkii*) with emphasis on biochemical characteristic and *in vitro* antibacterial effects of some plants extracts. World Applied Sciences Journal 6 (7), 868–879.

Hendrich, A.B., 2006. Flavonoid-membrane interactions: possible consequences for biological effects of some polyphenolic compounds. Acta Pharmacologica Sinica 27, 27–40.

Holt, J.G., Krieg, N.R., Sneathm, P.H.A., Staley, J.T., Williams, S.T., 1994. Bergey's Manual of Determinative Bacteriology, ninth ed. Williams and Williams, Baltimore, MD.

Ibrahim, T.A., Opawale, B.O., Oyinloye, J.M.A., 2011. Antibacterial activity of herbal extracts against multi drug resistant strains of bacteria from clinical origin. Life Sciences Leaflets 15, 490–498.

Kokate, C.K., 2001. Pharmacognosy, 16th ed. Nirali Prakashan, Mumbai, India.

Kong, J., Zhao, L., Xiao, H., Li, L., Jin, C., Li, B., 2009. Investigation of the anti-fungal activity of coptisine on *Candida albicans* growth by microcalorimetry combined with principal component analysis. Journal of Applied Microbiology 107, 1072–1080.

Kosolapov, D.B., Kuschk, P., Vainshtein, M.B., Vatsourina, A.V., Wiebner, A., Kastner, M., Muller, R.A., 2004. Microbial processes of heavy metal removal from carbon-deficient effluents in constructed wetlands. Engineering in Life Sciences 4, 403–411.

Kumar, C.S., Sarada, D.V.L., Gideon, T.P., Rengasamy, R., 2008. Antibacterial activity of three South Indian seagrasses, *Cymodocea serrulata*, *Halophila ovalis* and *Zostera capensis*. World Journal of Microbiology and Biotechnology 24 (9), 1989–1992.

Li, F., Hu, H., 2005. Isolation and characterization of a novel antialgal allelochemical from *Phragmites communis*. Applied and Environmental Microbiology 71 (11), 6545–6553.

Meda, A., Lamien, E., Romito, M., Millogo, J., Nacoulma, G., 2005. Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. Food Chemistry 91, 571–577.

National Committee for Clinical Laboratory Standards (NCCLS), 1999. Methods for determining bacteridical activity of antimicrobial agents, Proposed guideline. Approved guideline M26-A. National Committee for Clinical Laboratory Standards, Wayne, Pa.

Newman, D.J., Cragg, G.M., 2007. Natural products as source of new drugs over the last 25 years. Journal of Natural Products 70, 461–477.

Obeidat, M., Shatnawi, M., Al-alawi, M., Enas, A., Hanee, A., Masia, A., El-Quadah, J., Otr, I., 2012. Antimicrobial activity of crude extracts of some plant leaves. Research Journal of Microbiology 7, 59–67.

Okeke, I., Iroegbu, U., Eze, N., Okoli, S., Esimone, O., 2001. Evaluation of extracts of the root of *Landolphia owerrience* for antibacterial activity. Journal of Ethnopharmacology 78, 119–127.

Otludil, B., Agüloğlu, O.B., Demir, R., Tolan, V., Temel, H., 2005. The effect of extracellular and membrane in amylase production of the tetradentate Schiff base, its Mn(II), Ni(II), Cu(II) and Zn(II) complexes and metal ions in *Bacillus subtilis*. Biotechnology and Biotechnological Equipment 19 (2), 105–110.

Özbay, H., Alim, A., 2009. Antimicrobial activity of some water plants from the Northeastern Anatolian region of Turkey. Molecules 14, 321–328.

Pai, M.R., Acharya, L.D., Udupa, N., 2004. Evaluation of antiplaque activity of *Azadirachta indica* leaf extract gel a 6-week clinical study. Journal of Ethnopharmacology 90, 99–103.

Pandey, B.P., 1982, Taxonomy of angiosperms. In: Systematic Botany, fourth ed. Published by S. Chand & Co. Ltd, New Delhi, p. 10055.

Ratnam, K.V., Raju, R.V., 2008. *In vitro* antimicrobial screening of the fruit extracts of two *Syzygium* species (Myrtaceae). Advances in Biological Research 2, 17–20.

Ryan, T.A., Joiner, B.L., Ryan, B.F., 1976. Minitab Student Handbook. Duxbury Press, North Scituate, Boston, MA.

Salvador, N., Garreta, G., Lavelli, L., Ribera, A., 2007. Antimicrobial activity of Iberian macroalgae. Scientia Marina 71, 101–113.

SAS, 1996. SAS Users Guide: Statistical software package, version 2.5 developed by the SAS Institute, Inc., Cary, North Carolina, USA.

Shah, P.M., 2005. The need for new therapeutic agents: what is in the pipeline? Clinical Microbiology and Infection 11 (3), 36–42.

Shin, W.J., Lee, K.H., Park, M.H., Seong, B.L., 2010. Broad-spectrum antiviral effects of *Agrimonia palosa* extract on influenza viruses. Microbiology and Immunology 54, 11–19.

Sohail, M.N., Rasul, F., Karim, A., Kanwal, U., Attitalla, I.H., 2011. Plants as a source of natural antiviral agents. Asian Journal of Animal and Veterinary Advances 6 (12), 1125–1152.

Soetan, K.O., Oyekunle, M.A., Aiyelaagbe, O.O., Fafunso, M.A., 2006. Evaluation of the antimicrobial activity of saponins extract of *Sorghum Bicolor* L.. Moench African Journal of Biotechnology 5 (23), 2405–2407.

Sridevi, M., Rao, Kondala, Sathiraju, D., 2010. Sensitivity of bacteria isolated from Champavathi Estuary to some medicinal plants of Vizianagaram district, East coast of India. Drug Invention Today 2 (7), 366–368.

Täckholm, V., 1974. Student's Flora of Egypt. second ed. Cairo University Press, pp. 887.

Traub, H., Geipel, U., Leonhard, B., Bauer, D., 1998. Antibiotic susceptibility testing (agar disk diffusion and agar dilution) of clinical isolates of *Corynebacterium jeikeium*. Chemotherapy 44, 217–229.

Trombetta, D., Castelli, F., Sarpietro, G.M., Venuti, V., Cristani, M., Daniele, C., Saija, A., Mazzanti, G., Bisignano, G., 2005. Mechanisms of antibacterial action of three monoterpenes. Antimicrobial Agents and Chemotherapy 49 (6), 2474–2478.

Verma, H., Patil, P.R., Kolhapure, R.M., Gopalkrishna, V., 2008. Antiviral activity of the Indian medicinal plant extract *Swertia chirata* against herpes simplex viruses: a study by *in vitro* and molecular approach. Indian Journal of Medical Microbiology 26, 322–326.

Yi, Y., Yi, L., Yin, Y., Zhang, H., Wang, G., 2012. The antialgal activity of 40 medicinal plants against *Microcystis aeruginosa*. Journal of Applied Phycology 24 (4), 847–856.

Yisa, J., 2009. Phytochemical analysis and antimicrobial activity of *Scoparia Dulcis* and *Nymphaea lotus*. Australian Journal of Basic and Applied Sciences 3, 3975–3979.

Yu, J., Lei, J., Yu, H., Cai, X., Zou, G., 2004. Chemical composition and antimicrobial activity of the essential oil of *Scutellaria barbata*. Phytochemistry 65, 881–884.

Wang, J., Carnicella, S., Phamluong, K., Jeanblanc, J., Ronesi, J.A., Chaudhri, N., Janak, P.H., Lovinger, D.M., Ron, D., 2007. Ethanol induces long-term facilitation of NR2B-NMDA receptor activity in the dorsal striatum: implications for alcohol drinking behavior. The Journal of Neuroscience 27 (13), 3593–3602.